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COMPOSITIONS FOR INHALATION

This invention relates to methods and compositions for delivery of medically useful peptides and proteins.

Background of the Invention

Although the advent of recombinant DNA technology has resulted in a rapidly expanding list of peptide-based drugs, a major drawback of peptide-based therapy has acutely hampered realization of the full potential of this field: in general, peptide-based drugs cannot be orally administered in effective doses, since they are rapidly degraded by enzymes in the gastrointestinal tract before they can reach the bloodstream. Unless the polypeptide of interest can be altered to make it relatively resistant to such enzymes, the only practical method of delivering the drug is likely to be a parenteral route, such as by intravenous, intramuscular, or subcutaneous injection. Administration by other parenteral routes (e.g., by absorption across nasal, buccal or rectal membranes, or via the lung) has met with limited success.

Summary of the Invention

It has been found that when a peptide or protein (hereinafter collectively referred to as polypeptides) is combined with an appropriate absorption enhancer and is introduced into the lung in the form of a powder of appropriate particle size, it readily enters the pulmonary circulation by absorption through the layer of epithelial cells separating the alveoli from the pulmonary vasculature. This is conveniently accomplished by inhalation of the powder from a inhaler device which dispenses the correct dose of powdered polypeptide/enhancer in a particle size which maximizes deposition in the lower respiratory tract, as opposed to the mouth and throat. (For ease of reference, the polypeptide and enhancer are hereinafter collectively referred to as the "active compounds"). To accomplish this

preferential delivery into the lung, as much as possible of the active compounds should consist of particles having a diameter less than approximately 10 μm (e.g., between 0.01-10 μm, and ideally between 1-6 μm). In preferred embodiments, at least 50% (preferably at least 60%, more preferably at least 70%, still more preferably at least 80%, and most preferably at least 90%) of the total mass of active compounds which exits the inhaler device consists of particles within the desired diameter range.

The invention thus includes a pharmaceutical composition containing a mixture of active compounds (A) a pharmaceutically active polypeptide and (B) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory system (preferably the lungs) of a patient, the mixture being in the form of a dry powder suitable for inhalation, in which at least 50% of the total mass of active compounds (A) and (B) consists of primary particles having a diameter less than or equal to about 10 microns. The primary particles may be packaged as such, or may optionally be formed into agglomerates, which then are substantially deagglomerated prior to entry into the respiratory tract of the patient. The composition may of course contain other ingredients as needed, including other pharmaceutically active agents, other enhancers, and pharmacologically acceptable excipients such as diluents or carriers. Therefore, the therapeutic preparation of the present invention may contain only the said active compounds or it may contain other substances, such as a pharmaceutically acceptable carrier. This carrier may largely consist of particles having a diameter of less than about 10 microns so that at least 50 % of the resultant powder as a whole consists of optionally agglomerated primary particles having a diameter of less than about 10 microns; alternatively the carrier may largely consist of much bigger particles ("coarse particles"), so that an "ordered mixture" may be formed between the active compounds and the said carrier. In an ordered mixture, alternatively known as an interactive or adhesive mixture, fine drug particles (in this invention, the active compounds) are fairly evenly distributed over the surface of coarse excipient particles (in this invention, the pharmaceutically acceptable

carrier). Preferably in such case the active compounds are not in the form of agglomerates prior to formation of the ordered mixture. The coarse particles may have a diameter of over 20 microns, such as over 60 microns. Above these lower limits, the diameter of the coarse particles is not of critical importance so various coarse particle sizes may be used, if desired according to the practical requirements of the particular formulation. There is no requirement for the coarse particles in the ordered mixture to be of the same size, but the coarse particles may advantageously be of similar size within the ordered mixture. Preferably, the coarse particles have a diameter of 60 - 800 microns.

The polypeptide is preferably a polypeptide hormone other than insulin, such as vasopressin, glucagon, corticotropin (ACTH), gonadotrophin (luteinizing hormone, or LHRH), calcitonin, C-peptide of insulin, growth hormone (HG), growth hormone releasing hormone (GHRH), desmopressin, oxytocin, corticotropin releasing hormone (CRH), somatostatin analogs, gonadotropin agonist analogs (GnRHa), atrial natriuretic peptide (hANP), thyroxine releasing hormone (TRHrh), follicle stimulating hormone (FSH), prolactin, or melanocyte stimulating hormone (MSH). Alternatively, the polypeptide may be a growth factor; an interleukin; an antigen-binding fragment of an antibody; a targeted hybrid toxin; a polypeptide vaccine; an enzyme; an endorphin; or a soluble, ligand-binding fragment of a cellular receptor. The polypeptide preferably has a molecular weight of less than about 40 kD; more preferably, less than about 30 kD; and even more preferably, less than about 15 kD. Most preferably, the polypeptide has a molecular weight of less than 10kD.

The enhancer compound used in the compositions of the present invention can be any compound which enhances the absorption of the polypeptide through the epithelium of the alveoli, and into the systemic circulation. By "enhances absorption" is meant that the amount of polypeptide absorbed into the systemic circulation in the presence of the enhancer is significantly ($p<0.05$) higher than the amount absorbed in the absence of enhancer.

The amount of insulin absorbed according to the present invention is

preferably at least 150% of the amount absorbed in the absence of enhancer. In preferred embodiments, absorption of insulin is at least doubled, more preferably tripled, and most preferably quadrupled in the presence of the enhancer, compared to in its absence.

The enhancer is preferably a surfactant such as a salt of a fatty acid, a bile salt, or a phospholipid. The enhancer may be, for example, a sodium, potassium, or organic amine salt of the fatty acid, and the fatty acid is preferably capric acid or another fatty acid of 8-12 carbon atoms. The preferred enhancer is sodium caprate. The ratio of polypeptide to enhancer will preferably vary from about 9:1 to about 1:1. Although proportions of enhancer greater than 1:1 would presumably enhance uptake as well as or better than lower proportions, it is believed that the amount of enhancer used should be no higher than necessary to acheive the desired level of enhancement, since excess enhancer may trigger unwanted side effects, such as local irritation.

Also within the invention is a method of administering systemically a pharmaceutically active polypeptide, by causing a patient to inhale the pharmaceutical composition of the invention, wherein at least 50% of the total mass of the active compounds at the point of entry to the respiratory tract of the patient consists of particles having a diameter less than or equal to about 10 microns. This is preferably accomplished by the use of an inhaler device from which the patient inhales the powder. Where the powdered composition is in the form of agglomerates of primary particles, the device is preferably configured to induce substantial deagglomeration of the agglomerates upon inhalation of the powder from the device by the patient, so that the majority of the agglomerates break down into particles having a diameter less than or equal to about 10 microns, prior to entry of the powder into the respiratory system of the patient. This deagglomeration would occur inside the device, and is typically induced by the air turbulence created in the device by the force of inhalation. Agglomerates are in general preferably not formed in the ordered mixture. In the case of an ordered mixture, the active compounds should be released from the large particles

preferably upon inhalation, either by mechanical means in the inhaler device or simply by the action of inhalation, or by other means, the active compounds then being deposited in the lower respiratory tract and the carrier particles in the mouth.

The inhaler device is preferably a single dose dry powder inhaler, but may alternatively be a multi dose dry powder inhaler.

The invention also includes processes for the manufacture of a pharmaceutical composition suitable for administration by inhalation. In one such process, a solution is first provided in which are dissolved (a) a pharmaceutically active polypeptide and (b) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory tract of a patient. The solvent is then removed from the solution to yield a dry solid containing the polypeptide and the enhancer, and the dry solid is pulverized to produce a powder. A second such process involves dry mixing (a) a pharmaceutically active polypeptide and (b) an enhancer compound, and micronizing the obtained mixture. Yet a third suitable process includes the steps of providing a first micronized preparation containing a polypeptide and a second micronized preparation containing an enhancer compound, and mixing the two micronized preparations together. When a carrier is to be included other than when an ordered mixture is desired, this may be added to the solution, or to the dry-mixture of the pharmaceutically active polypeptide prior to micronization, or micronised carrier may be dry mixed with the other micronised components. In producing an ordered mixture, micronised polypeptide and enhancer are mixed with a suitable carrier.

Brief Description of the Drawings

Fig. 1 is a graph illustrating the effects of different concentrations of sodium caprate enhancer on the transport of a marker compound (mannitol) through a monolayer of cultured epithelial cells.

Fig. 2 is a graph illustrating the effects of different concentrations of sodium caprate enhancer on the transport of a marker compound (mannitol)

through a monolayer of cultured epithelial cells, in the presence of insulin (sodium caprate:insulin 1:3 by weight).

Detailed Description

Some of the preferred embodiments of the invention are generally described below.

The Polypeptide

The polypeptide to be delivered by the method of the invention can be any medically or diagnostically useful peptide or protein for which systemic delivery is desired. Suitably the polypeptide may be a peptide hormone such as insulin, vasopressin, glucagon, corticotropin (ACTH), gonadotrophin (luteinizing hormone, or LHRH), calcitonin, C-peptide of insulin, growth hormone (HG), growth hormone releasing hormone (GHRH), desmopressin, oxytocin, corticotropin releasing hormone (CRH), somatostatin analogs, gonadotropin agonist analogs (GnRHa), atrial natriuretic peptide (hANP), thyroxine releasing hormone (TRHrh), follicle stimulating hormone (FSH), prolactin, and melanocyte stimulating hormone (MSH).

Other possible polypeptides include growth factors such as erythropoietin, nerve growth factors, bone morphogenic proteins, insulin-like growth factors, the interleukins, nerve growth factor (NGF), epidermal growth factors (EGF), platelet-derived growth factor (PDGF), hematopoietic colony-stimulating factors (such as CSF-1, granulocyte-macrophage (GM)-CSF, and macrophage (M)-CSF), transforming growth factor β (TGFβ), the interferons (α, β, and γ), tumor necrosis factor (TNF) α and β, superoxide dismuthase (SOD), lactoferrin, acidic isoferritin, activin, and inhibin; antigen-binding fragments of antibodies (e.g., FAB fragments); immunotoxins and other targeted hybrid toxins, both chemically conjugated and genetically engineered (e.g., the bacterial toxin-based hybrids disclosed in PCT International Publication No. WO83/03971); polypeptide vaccines (e.g., diphtheria toxin genetically engineered to destroy its

enzymatic activity and therefore its cytotoxicity); and soluble, ligand-binding fragments of the extracellular domains of cellular receptors (e.g., soluble CD4 for combatting HIV infection). Any given peptide or protein can be readily tested by one of ordinary skill for use in the methods of the invention by combining it with an absorption-enhancing agent as described herein, and testing the combination in the *in vivo* or *in vitro* assays described below. It is expected that most if not all polypeptides of small to medium size (e.g., up to approximately 40 kD, preferably up to 30 kD, more preferably up to 15 kD, and most preferably up to 10 kD), relatively high water solubility, and an isoelectric point between approximately pH 3 and pH 8 can be effectively delivered by the methods of the invention.

The Enhancer

The use of an absorption enhancer is of critical importance, as the polypeptide alone is poorly absorbed through the lung. The enhancer used can be any of a number of compounds which act to enhance absorption through the layer of epithelial cells lining the alveoli of the lung, and into the adjacent pulmonary blood supply. The enhancer can accomplish this by any of several possible mechanisms:

(1) Enhancement of the paracellular permeability of the polypeptide by inducing structural changes in the tight junctions between the epithelial cells.

(2) Enhancement of the transcellular permeability of the polypeptide by interacting with or extracting protein or lipid constituents of the membrane, and thereby perturbing the membrane's integrity.

(3) Interaction between enhancer and the polypeptide which increases the solubility of the polypeptide in aqueous solution. This may occur by preventing formation of polypeptide aggregates (dimers, trimers, hexamers), where such aggregates are otherwise prone to occur, or by solubilizing the polypeptide molecules in enhancer micelles.

(4) Decreasing the viscosity of, or dissolving, the mucus barrier lining the alveoli and passages of the lung, thereby exposing the epithelial surface

for direct absorption of the polypeptide.

Enhancers may function by only a single mechanism set forth above, or by two or more. An enhancer which acts by several mechanisms is more likely to promote efficient absorption of the polypeptide than one which employs only one or two. For example, surfactants are a class of enhancers which are believed to act by all four mechanisms listed above. Surfactants are amphiphilic molecules having both a lipophilic and a hydrophilic moiety, with varying balance between these two characteristics. If the molecule is very lipophilic, the low solubility of the substance in water may limit its usefulness. If the hydrophilic part overwhelmingly dominates, however, the surface active properties of the molecule may be minimal. To be effective, therefore, the surfactant must strike an appropriate balance between sufficient solubility and sufficient surface activity.

Another surfactant property that may be of importance is the net charge of the surfactant at the pH value in the lung (approximately 7.4). For example, insulin (which has an isoelectric point of 5.5) has a negative net charge at pH 7.4. This results in an electrostatic repulsion between insulin molecules, which in turn prevents aggregation and thereby increases the solubility. If the surfactant also is negatively charged, yet can interact with insulin by, for example, hydrophobic interactions, additional repulsion among the insulin molecules will occur. Therefore, an anionic surfactant will possess the additional advantage (compared to those having neutral or net positive charge at physiological pH) of enhancing absorption by helping stabilize insulin, or other polypeptides which behave similarly to insulin, in the monomeric state.

One very promising type of enhancer is the salt of a fatty acid. It has been found that the sodium salt of a saturated fatty acid having a carbon chain length of 10 (i.e., sodium caprate) performs very well in the method of the invention. If the chain length is shorter, the surface activity of the surfactant may be too low, and if the chain length is longer, decreased solubility of the fatty acid salt in water limits its usefulness.

A counterion other than sodium may increase the solubility of the

saturated fatty acid salt in water, such that a carbon length greater than 10 would prove even more advantageous than does sodium caprate. Since salts of unsaturated fatty acids are more water soluble than salts of saturated fatty acids, the former can have a longer chain length than the latter and still maintain the solubility necessary for a successful enhancer of insulin absorption.

All of the bile salts and bile salt derivatives tested (sodium salts of ursodeoxycholate, taurocholate, glycocholate, and taurodihydrofusidate) effectively enhance polypeptide absorption in the lung.

Of the phospholipids tested, a single-chain phospholipid (lysophospatidylcholine) was an effective enhancer, while two double-chain phospholipids (dioctanoylphosphatidylcholine and didecanoylphosphatidylcholine) were not. This may be explained by the fact that the double-chain phospholipids are much less soluble in water than their single-chain counterparts.

One glycoside, octylglucopyranoside, was not clearly effective as an enhancer of insulin absorption in the lung. It is believed that this is because it is not negatively charged at physiological pH, and so does not promote insulin solubility as does an anionic surfactant. It may be more effective with other polypeptides.

Another class of potentially useful surfactants are the naturally occurring surfactants such as salts of glycyrrhizine acid, saponin glycosides and acyl carnitines.

For ionic enhancers (e.g., the anionic surfactants described above), the nature of the counterion may be important. The particular counterion selected may influence the powder properties, solubility, stability, hygroscopicity, and local/systemic toxicity of the enhancer or of any formulation containing the enhancer. It may also affect the stability and/or solubility of the polypeptide with which it is combined. In general, it is expected that monovalent metallic cations such as sodium, potassium, lithium, rubidium, and cesium will be useful as counterions for anionic enhancers. Ammonia and organic amines form another class of cations that is expected to be appropriate for use with anionic enhancers

having a carboxylic acid moiety. Examples of such organic amines include ethanolamine, diethanolamine, triethanolamine, 2-amino-2-methylethylamine, betaines, ethylenediamine, N,N-dibensylethylenetetraamine, arginine, hexamethylenetetraamine, histidine, N-methylpiperidine, lysine, piperazine, spermidine, spermine, and tris(hydroxymethyl)aminomethane.

Since effective enhancement of polypeptide absorption in the lung was observed for a number of the enhancers tested, it is expected that many more will be found which also function in this manner. For example, the cyclodextrins (either naturally occurring or synthetic modifications) are known to enhance significantly the nasal absorption of a polypeptide, and may function similarly in the lung. Starch microspheres effectively enhance the bioavailability of insulin delivered via the nasal membranes and were tested as an enhancer in the methods of the invention. Although they proved to be of little use for delivery via the pulmonary route in the animal model utilized herein, it is thought that this was mainly due to technical difficulties which, if overcome, may lead to successful delivery via the pulmonary route.

Chelators are a class of enhancers that are believed to act by binding calcium ions. Since calcium ions help maintain the dimensions of the space between cells and additionally reduce the solubility of some polypeptides, binding of these ions would in theory both increase the solubility of such polypeptides, and increase the paracellular permeability of the polypeptides. Although one chelator tested, the sodium salt of ethylenediaminetetraacetic acid (EDTA), was found to be ineffective in enhancing absorption of insulin in the rat model tested, other calcium ion-binding chelating agents may prove to be more useful.

Other substances with known absorption-enhancing properties, or with physical characteristics which make them likely candidates for use in the method of the invention, can be readily tested by one of ordinary skill in the assays described herein. It is possible that a combination of two or more enhancer substances also give satisfactory results. The use of such a combination in the method of the invention is considered to be within the invention.

An enhancer useful in the methods of the invention will combine effective enhancement of polypeptide absorption with (1) lack of toxicity in the concentrations used and (2) good powder properties, i.e., lack of a sticky or waxy consistency in the solid state. Toxicity of a given substance can be tested by standard means, such as the MTT assay as for example described in Int. J. Pharm. 65 (1990) 249-259. The powder properties of a given substance may be ascertained from published data on the substance, or empirically.

The amount of pharmaceutically active polypeptide absorbed according to the present invention can be significantly higher than the amount absorbed in the absence of enhancer.

Proportions of insulin and enhancer

The relative proportions of polypeptide and enhancer may be varied as desired. Sufficient enhancer must be present to permit efficient absorption of the inhaled polypeptide; however, the amount of enhancer should be kept as low as possible in order to minimize the risk of adverse effects caused by the enhancer. Experiments carried out with sodium caprate combined in various ratios with insulin as the polypeptide, indicate that for this particular compound, acceptable absorption of polypeptide requires that a minimum of 20-25% of the polypeptide/enhancer mixture be enhancer. While each particular polypeptide/enhancer combination must be tested to determine the optimal proportions, it is expected that to achieve acceptable absorption of the polypeptide, at least 10% (preferably at least 15%, and more preferably at least 20%) of the polypeptide/enhancer mixture must be enhancer; for most types of enhancers, the proportion of enhancer necessary to achieve satisfactory results will probably be between 10 and 50%. The preferred ratio for each polypeptide/enhancer (or polypeptide/enhancer/diluent) combination can be readily determined by one of ordinary skill in the art of pharmacology by standard methods, based on such criteria as efficient, consistent delivery of the optimal dosage, minimization of side effects, and acceptable rate of absorption.

No further ingredients are needed for the action of the preparation, but may be included if desired. For example, the amount of powder which constitutes a single dose of a given polypeptide/surfactant combination could be increased (e.g., for use in an inhaler apparatus which by design requires a large powder volume per dose) by diluting the powder with pharmaceutically acceptable diluents, such as lactose glucose or mannitol. Other additives may be included to facilitate processing or to improve the powder properties or stability of the preparation. A flavoring agent could be added so that the proportion of the powder which is inevitably deposited in the mouth and throat would serve to give the patient positive feedback that a dose had been delivered from the inhaler device. Any such additive should have the following properties: (a) it is stable and does not disadvantageously affect the stability of the polypeptide and enhancer; (b) it does not disadvantageously interfere with absorption of the polypeptide; (c) it has good powder properties, as that term is understood in the pharmaceutical arts; (d) it is not hygroscopic; and (e) it has no adverse effects in the airways in the concentrations used. Useful types of such additives include mono-, di-, and polysaccharides, sugar alcohols, and other polyols: for example, lactose, glucose, mannitol, and starch. Such additives may constitute anywhere from 0% (i.e., no additive) to nearly 100% of the total preparation.

In a preferred embodiment, this invention provides a therapeutic preparation of a pharmaceutically active polypeptide and a substance which enhances the absorption of said polypeptide in the lower respiratory tract, which preparation is in the form of a dry powder preparation suitable for inhalation of which at least 50% by mass consists of (a) particles having a diameter of less than about 10 microns or (b) agglomerates of said particles; in another preferred embodiment, the invention provides a therapeutic preparation comprising a pharmaceutically active polypeptide, a substance which enhances the absorption of insulin in the lower respiratory tract, and a pharmaceutically acceptable carrier, which preparation is in the form of a dry powder suitable for inhalation of which at least 50% by mass consists of (a) particles having a diameter of less than about 10

microns, or (b) agglomerates of said particles; and in a further preferred embodiment this invention provides a therapeutic preparation comprising active compounds (A) a pharmaceutically active polypeptide and (B) a substance which enhances the absorption of said polypeptide in the lower respiratory tract, wherein at least 50 % of the total mass of active compounds (A) and (B) consists of particles having a diameter of less than about 10 microns, and a pharmaceutically acceptable carrier, which preparation is in the form of a dry powder preparation suitable for inhalation in which an ordered mixture may be formed between the active compounds and the pharmaceutically acceptable carrier.

The described powder preparation could be manufactured in several ways, using conventional techniques In many cases, the purified polypeptide can be obtained from commercial sources. Alternatively, the polypeptide of interest can be purified from a naturally occurring source using standard biochemical techniques, or can be obtained by expression of prokaryotic or eukaryotic cells genetically engineered to contain a nucleotide sequence which encodes the polypeptide and has appropriate expression control sequences linked thereto (including a transgenic animal engineered to manufacture the desired peptide or protein, for example in its milk). Such methods are standard in the art (e.g., see Sambrook et al., Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Peptides (i.e., polypeptides having 30 or fewer amino acid residues) can be readily synthesized by known chemical means.

Absorption enhancers as described above are also generally available from commercial sources, or can be manufactured using published methods. For ionic enhancers, the counterion associated with the enhancer can be replaced with another, if desired, using standard ion exchange techniques.

In manufacturing of the described powder preparation it will in general be necessary to micronize the powder in a suitable mill, e.g. a jet mill, at some point in the process, in order to produce primary particles in a size range appropriate for maximal deposition in the lower respiratory tract (i.e., under

10 μm). For example, one can dry mix polypeptide and enhancer powders, and then micronize the substances together; alternatively, the substances can be micronized separately, and then mixed.

It is also possible first to dissolve the components in a suitable solvent, e.g. water, to obtain mixing on the molecular level. This procedure also makes it possible to adjust the pH-value to a desired level, for instance to improve absorption of the polypeptide. The pharmaceutically accepted limits of pH 3.0 to 8.5 for inhalation products must be taken into account, since products with a pH outside these limits may induce irritation and constriction of the airways. To obtain a powder, the solvent must be removed by a process which retains the polypeptide's biological activity. Suitable drying methods include vacuum concentration, open drying, spray drying, and freeze drying. Temperatures over 40°C for more than a few minutes should generally be avoided, as some degradation of the certain polypeptides may occur. Following the drying step, the solid material can, if necessary, be ground to obtain a coarse powder, then, if necessary, micronized.

If desired, the micronized powder can be processed to improve the flow properties, e.g., by dry granulation to form spherical agglomerates with superior handling characteristics, before it is incorporated into the intended inhaler device. In such a case, the device would be configured to ensure that the agglomerates are substantially deagglomerated prior to exiting the device, so that the particles entering the respiratory tract of the patient are largely within the desired size range. Where an ordered mixture is desired, the active compound may be processed, for example by micronisation, in order to obtain, if desired, particles within a particular size range. The carrier may also be processed, for example to obtain a desired size and desirable surface properties, such as a particular surface to weight ratio, or a certain ruggedness, and to ensure optimal adhesion forces in the ordered mixture. Such physical requirements of an ordered mixture are well known, as are the various means of obtaining an ordered mixture which fulfills the said requirements, and may be determined easily by the skilled person according to

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the particular circumstances.

A preferred inhalation apparatus would have the following design characteristics: protection of the powder from moisture and no risk from occasional large doses; in addition as many as possible of the following are desired: protection of the powder from light; ; high respirable fraction and high lung deposition in a broad flow rate interval; low deviation of dose and respirable fraction; low retention of powder in the mouthpiece - this is particularly important for a multidose inhaler, where polypeptide retained in the mouthpiece could degrade and then be inhaled together with subsequent doses; low adsorption to the inhaler surfaces; flexibility in dose size; and low inhalation resistance. The inhaler is preferably a single dose inhaler although a multi dose inhaler, such as a multi dose, breath actuated, dry powder inhaler for multiple use, may also be employed. Peferably the inhaler used is a unit dose, breath actuated, dry powder inhaler for single use.

A number of dry powder formulations containing a polypeptide and various enhancers have been prepared and tested in an *in vivo* assay, and are described below. Also described is an *in vitro* assay useful for testing polypeptide/enhancer combinations.

Example 1

9.75 g of human insulin and 250 ml water are added to a beaker. The pH is lowered to 3.4 with 1 M HCl and then raised to 7.4 with 1 M NaOH in order to dissolve the insulin. 3.25 g sodium caprate is added and the pH is again adjusted to 7.4. The solution is stirred, and when the solution is clear or weakly opalescent, it is concentrated by evaporation at 37°C in about 2 days. The obtained solid cake is crushed and then sieved through a 0.5 mm sieve. The powder is micronized in a jet mill to produce particles with a mass median diameter of about 2 μm. This micronized powder, containing 75% insulin and 25% sodium caprate by weight, is filled into an appropriate inhalation apparatus, and delivered to animals. Blood glucose and plasma insulin values are measured at

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specified time intervals.

The results from an inhalation study in two dogs are summarized in the tables below.

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TABLE I

Blood sample time after end of expo (minutes)	Blood glucose (mmol/L)	Insulin conc (μU/ml)
before	3.9	6.70
0.5	3.6	120.66
5	2.8	194.47
10	2.6	195.39
20	n.d.	139.74
22.5	1.6	n.d.
31	2.0	73.42
45	1.7	47.49
59.5	1.7	36.21
89.5	2.3	19.28
120	3.0	14.58
240	4.5	5.28

n.d. = not determined

TABLE II

Blood sample time after end of expo (minutes)	Blood glucose (mmol/L)	Insulin conc (μU/ml)
before	3.9	44.84
3	4.2	165.10
6	4.3	158.28
12	3.9	n.d.
14	n.d.	180.72
19	3.0	133.75
30	2.7	143.71
45	2.5	91.62
60	2.4	66.70
90	2.7	38.58
122	3.7	29.15
241	4.1	n.d.
242.5	n.d.	19.76

n.d. = not determined

It is obvious from the above tables that the formulation markedly increases the plasma level of insulin and decreases the blood glucose. The peak value for plasma insulin and the minimal value for blood glucose are reached after approximately 20 and 60 minutes, respectively.

Example 2

Each of the compounds listed in Table III was tested for its ability to enhance uptake of a polypeptide (insulin) in a rat model. Various forms of insulin were employed in the different trials: recombinant human, semisynthetic human or bovine. Each formulation was prepared as above, drying and processing the

insulin/enhancer or insulin/enhancer/lactose solution to produce an inhalable powder. The powder was administered to rats by inhalation, and the blood glucose levels of the rats were subsequently monitored as a measure of insulin uptake. These levels were compared to the corresponding values obtained from rats which had inhaled insulin formulations without enhancer.

The same *in vivo* model system could be used to test any given peptide or protein for usefulness in the methods of the invention, by delivering by the same inhalation method a formulation containing the desired peptide or protein combined with an enhancer, and assaying for the concentration of the desired peptide or protein in the systemic circulation of the test animal (e.g., by standard immonoassays or biochemical assays as appropriate for the given peptide or protein).

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TABLE III

Substance	Enhancer:Insulin:lactose	Effect
Octylglucopyranoside	4:4:92	(+)
Sodium ursodeoxycholate	4:4:92	+
Sodium taurocholate	4:4:92	+
Sodium glycocholate	4:4:92	+
Lysophosphatidylcholine	4:4:92	+
Dioctanoylphosphatidylcholine	2:4:94	(+)
Didecanoylphospatidylcholine	4:4:94	-
Sodium taurodihydrofusidate	2:4:94	+
Sodium caprylate	25:75:0	-
Sodium caprate	10:90:0	(+)
Sodium caprate	17.5:82.5:0	(+)
Sodium caprate	25:75:0	+
Sodium caprate	4:4:92	+
Sodium laurate	25:75:0	(+)
Potassium oleate	4:4:92	+

+ effect, i.e. enhancer gives a significant defect in blood glucose level.

- no or very small effect.

(+) effect not clear, i.e. no definate assessment could be made.

Example 3

A standard *in vitro* assay utilizing an epithelial cell line, CaCo-2 (available through the American Type Culture Collection (ATCC), Rockville, MD, USA), has been developed to assess the ability of various enhancer compounds to promote transport of insulin and other markers across an epithelial cell monolayer, as a model for the epithelial cell layer which functions in the lung to separate the alveolus from the pulmonary blood supply. In this assay, the enhancer and polypeptide or other marker are dissolved in aqueous solution at various proportions and/or concentrations, and applied to the apical side of the cell monolayer. After 60 min incubation at 37°C and 95% RH (relative humidity), the amount of the marker on the basolateral side of the cells is determined, e.g by use of a radioactively labelled marker. For the enhancer tested, sodium caprate, the amount of marker (mannitol, MW 360) which appears on the basolateral side is dependent upon the concentration of enhancer used, at least up to 16 mM sodium caprate (Fig. 1). This is true even when insulin is added to the enhancer/mannitol mixture (1:3 sodium caprate:insulin, by weight) (Fig. 2). This concentration of sodium caprate (16 mM) was also found to promote absorption across the cell monolayer of two low molecular weight peptides, insulin (MW 5734) and vasopressin (MW 1208). The amount of insulin which passed across the monolayer doubled in the presence of 16 mM sodium caprate, compared to the amount in the absence of any enhancer; the amount of vasopressin which was absorbed across the monolayer increased 10-15 times compared to the amount in the absence of any enhancer. In contrast, no increase in transport rate was observed for larger proteins such as cytochrome C (MW 12,300), carbonic anhydrase (MW 30,000) and albumin (MW 69,000) when tested at up to 16 mM sodium caprate. It is expected that at higher concentrations of sodium caprate, the permeability of the cells will be further increased, permitting the transport of larger polypeptides; however, the potential cytotoxicity of sodium caprate may prevent the use of substantially higher concentrations of this particular enhancer.

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This *in vitro* model of epithelial cell permeability can be used as a screening tool for rapidly testing any desired polypeptide/enhancer combination for usefulness in the methods of the invention.

What is claimed is:

1. A pharmaceutical composition, comprising a mixture of active compounds (A) a pharmaceutically active polypeptide and (B) an enhancer compound which enhances the systemic absorption of said polypeptide in the lower respiratory tract of a patient, said mixture being in the form of a dry powder for inhalation, in which at least 50% of the total mass of active compounds consists of primary particles having a diameter less than or equal to about 10 microns, said primary particles optionally being formed into agglomerates.

2. A pharmaceutical composition as claimed in claim 1, additionally comprising a pharmaceutically acceptable carrier, which comprises either

(a) particles having a diameter of less than about 10 microns, such that at least 50 % of the resultant powder consists of optionally agglomerated primary particles having a diameter of less than about 10 microns; or

(b) coarse particles, such that an ordered mixture is formed between the active compounds and the said carrier.

3. The composition of claim 1, wherein said polypeptide is a polypeptide hormone.

4. The composition of claim 3, wherein said hormone is vasopressin, glucagon, calcitonin, corticotropin, gonadotrophin, C-peptide of insulin, growth hormone (HG), growth hormone releasing hormone (GHRH), desmopressin, oxytocin, corticotropin releasing hormone (CRH), somatostatin analogs, gonadotropin agonist analogs (GnRHa), atrial natriuretic peptide (hANP), thyroxine releasing hormone (TRHrh), follicle stimulating hormone (FSH), prolactin, or melanocyte stimulating hormone (MSH).

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5. The composition of claim 1, wherein said polypeptide is a growth factor; an interleukin; an antigen-binding fragment of an antibody; a targeted hybrid toxin; a polypeptide vaccine; an enzyme; superoxide dismuthase (SOD); an endorphin; or a soluble, ligand-binding fragment of a cellular receptor.

6. The composition of claim 1, wherein said polypeptide has a molecular weight of less than 40 kD.

7. The composition of claim 1, wherein said polypeptide has a molecular weight of less than 15 kD.

8. The composition of claim 1, wherein said enhancer compound is a surfactant.

9. The composition of claim 8, wherein said surfactant is a salt of a fatty acid.

10. The composition of claim 9, wherein said fatty acid has 8-12 carbon atoms.

11. The composition of claim 10, wherein said fatty acid is capric acid.

12. The composition of claim 11, wherein said surfactant is sodium caprate.

13. An inhaler device containing the composition of claim 1.

14. The inhaler device of claim 13, wherein said composition is in the form of said agglomerates, said device being configured to induce the majority

of said agglomerates to break down into particles having a diameter less than or equal to about 10 microns, upon inhalation of said agglomerates from said device.

15. The inhaler device of claim 14, which inhaler device is a unit dose, breath actuated, dry powder inhaler for single use.

16. The inhaler device of claim 15, which inhaler device is a multi dose, breath actuated, dry powder inhaler for multiple use.

17. A method for systemic administration of a pharmaceutically active polypeptide, comprising

providing a composition comprising a mixture of active compounds (A) a pharmaceutically active polypeptide and (B) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory tract of a patient, said composition being in the form of a dry powder; and

causing said patient to inhale said composition; provided that the diameter of the particles of the active compounds at the point they enter the respiratory tract of the patient is less than or equal to about 10 microns.

18. The method of claim 17, wherein said composition is inhaled from an inhaler device which contains said powder in the form of agglomerates of said particles, said agglomerates being substantially deagglomerated prior to entering the respiratory tract of said patient.

19. A process for the manufacture of a pharmaceutical composition suitable for administration by inhalation, comprising

providing a solution in which are dissolved (a) a pharmaceutically active polypeptide and (b) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory tract of a patient;

removing the solvent from said solution to yield a dry solid

comprising said polypeptide and said enhancer compound; and

pulverizing said dry solid to produce a powder.

20. A process for the preparation of a pharmaceutical composition suitable for administration by inhalation, comprising

dry mixing (a) a pharmaceutically active polypeptide and (b) an enhancer compound which enhances the absorption of the polypeptide in the lower respiratory tract of a patient; and

micronizing the obtained mixture.

21. A process for the manufacture of a pharmaceutical composition suitable for administration by inhalation, comprising

providing a first micronized preparation comprising a polypeptide and a second micronized preparation comprising an enhancer compound which enhances the absorption of the polypeptide in the lung of a patient; and

mixing said first and second micronized preparations.

22. Use of an enhancer in the preparation of an inhalable dry powder preparation of polypeptide, with enhanced systemic absorption of said polypeptide in the lower respiratory tract, in which at least 50% of total mass of polypeptide and enhancer consists of (1) particles having a diameter of 10 microns or less, or (2) agglomerates of said particles.

23. Use according to claim 22, wherein the polypeptide is a polypeptide hormone.

24. Use according to claim 23, wherein said hormone is vasopressin, glucagon, calcitonin, corticotropin, gonadotrophin, C-peptide of insulin, growth hormone (HG), growth hormone releasing hormone (GHRH), desmopressin, oxytocin, corticotropin releasing hormone (CRH), somatostatin analogs,

gonadotropin agonist analogs (GnRHa), atrial natriuretic peptide (hANP), thyroxine releasing hormone (TRHrh), follicle stimulating hormone (FSH), prolactin, or melanocyte stimulating hormone (MSH).

25. Use according to claim 24, wherein the enhancer is a surfactant.

26. Use according to claim 25, wherein the enhancer is a salt of a fatty acid.

27. Use according to claim 26, wherein the enhancer is sodium caprate.

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COMPOSITIONS FOR INHALATION

Abstract of the Disclosure

Pharmaceutical compositions containing a mixture of a pharmaceutically active polypeptide and an enhancer compound which enhances the systemic absorption of the polypeptide in the lung of a patient, the mixture being in the form of a dry powder, in which at least 50% of the total mass of polypeptide and enhancer consists of primary particles having a diameter less than or equal to about 10 microns, the primary particles optionally being formed into agglomerates; and methods of delivering such compositions by inhalation.

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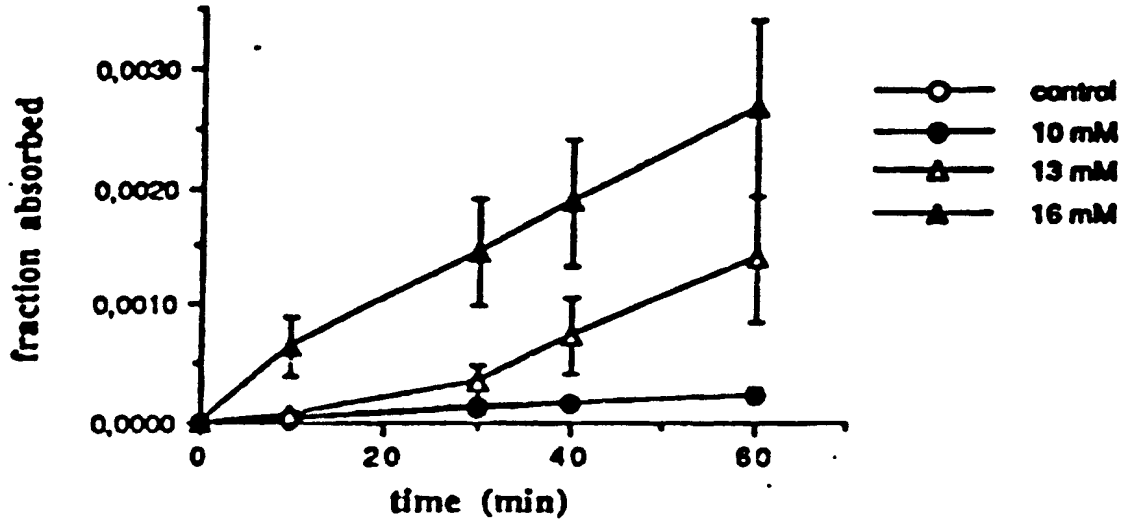
Fig.1



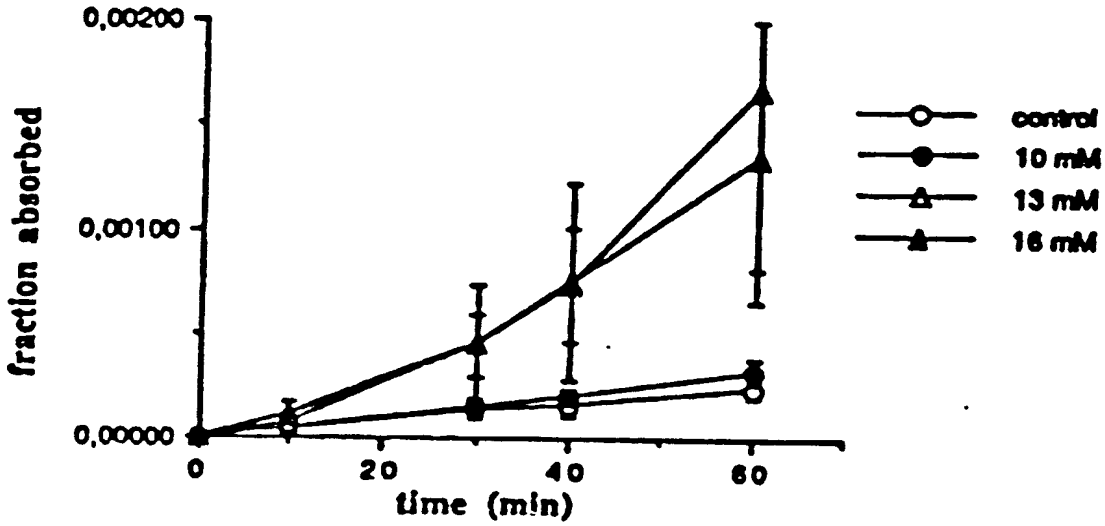


Fig. 2